



57 1000

SEQUENCE LISTING

<110> Case, Casey Christopher
Wolffe, Alan
Urnov, Fyodor
Lai, Albert
Snowden, Andrew
Tan, Siyuan
Gregory, Philip

<120> MODULATION OF ENDOGENOUS GENE EXPRESSION IN CELLS

<130> 8325-0002.21 / S2-US5

<140> 09/942,087
<141> 2001-08-28

<150> 09/229,037
<151> 1999-01-12

<160> 43

<170> PatentIn Ver. 2.0

<210> 1
<211> 25
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:exemplary motif
of C2H2 class of zinc finger proteins (ZFP)

<220>

<221> MOD_RES
<222> (2)..(3)
<223> Xaa = any amino acid

<220>

<221> MOD_RES
<222> (4)..(5)
<223> Xaa = any amino acid, may be present or absent

<220>

<221> MOD_RES
<222> (7)..(18)
<223> Xaa = any amino acid

<220>

<221> MOD_RES
<222> (20)..(22)
<223> Xaa = any amino acid

<220>

<221> MOD_RES
<222> (23)..(24)
<223> Xaa = any amino acid, may be present or absent

<400> 1

Cys Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

1

5

10

15

Xaa Xaa His Xaa Xaa Xaa Xaa Xaa His
20 25
<210> 2
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:ZFP target site
with two overlapping D-able subsites

<220>
<221> modified_base
<222> (1)..(2)
<223> n = g,a,c or t

<220>
<221> modified_base
<222> (5)
<223> n = g,a,c or t

<220>
<221> modified_base
<222> (8)
<223> n = g,a,c or t

<220>
<221> modified_base
<222> (9)
<223> n = a,c or t; if g, then position 10 cannot be g
or t

<220>
<221> modified_base
<222> (10)
<223> n = a or c; if g or t, then position 9 cannot be g

<400> 2
nngkngknnn

10

<210> 3
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:ZFP target site
with three overlapping D-able subsites

<220>
<221> modified_base
<222> (1)..(2)
<223> n = g,a,c or t

<220>
<221> modified_base
<222> (5)

<223> n = g,a,c or t

<220>
<221> modified_base
<222> (8)
<223> n = g,a,c or t

<400> 3
nngkngkngk

10

<210> 4
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:linker

<400> 4
Asp Gly Gly Gly Ser
1 5

<210> 5
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:linker

<400> 5
Thr Gly Glu Lys Pro
1 5

<210> 6
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:linker

<400> 6
Leu Arg Gln Lys Asp Gly Glu Arg Pro
1 5

<210> 7
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:linker

<400> 7

Gly Gly Arg Arg
1

<210> 8
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:linker

<400> 8
Gly Gly Gly Gly Ser
1 5

<210> 9
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:linker

<400> 9
Gly Gly Arg Arg Gly Gly Ser
1 5

<210> 10
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:linker

<400> 10
Leu Arg Gln Arg Asp Gly Glu Arg Pro
1 5

<210> 11
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:linker

<400> 11
Leu Arg Gln Lys Asp Gly Gly Ser Glu Arg Pro
1 5 10

<210> 12
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:linker

<400> 12
 Leu Arg Gln Lys Asp Gly Gly Gly Ser Gly Gly Ser Glu Arg Pro
 1 5 10 15

<210> 13
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:ZFP target site
 region surrounding initiation site of vascular
 endothelial growth factor (VEGF) gene containing
 two 9-base pair target sites

<220>
 <221> protein_bind
 <222> (4)..(12)
 <223> upstream 9-base pair ZFP VEGF1 target site

<220>
 <221> protein_bind
 <222> (14)..(22)
 <223> downstream 9-base pair ZFP VEGF3a target site

<400> 13
 agcggggagg atcgcggagg cttgg 25

<210> 14
 <211> 298
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:VEGF1 ZFP
 construct targeting upstream 9-base pair target
 site in VEGF promoter

<220>
 <221> CDS
 <222> (2)..(298)
 <223> VEGF1

<400> 14
 g gta ccc ata cct ggc aag aag aag cag cac atc tgc cac atc cag ggc 49
 Val Pro Ile Pro Gly Lys Lys Gln His Ile Cys His Ile Gln Gly
 1 5 10 15

tgt ggt aaa gtt tac ggc aca acc tca aat ctg cgt cgt cac ctg cgc 97
 Cys Gly Lys Val Tyr Gly Thr Ser Asn Leu Arg Arg His Leu Arg
 20 25 30

tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt ggt 145
 Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly

35	40	45	
aaa cgc ttc acc cgt tcg tca aac ctg cag cgt cac aag cgt acc cac			193
Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His			
50	55	60	
acc ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc atg			241
Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met			
65	70	75	80
cgt agt gac cac ctg tcc cgt cac atc aag acc cac cag aat aag aag			289
Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys			
85	90	95	
ggt gga tcc			298
Gly Gly Ser			

<210> 15
<211> 99
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:VEGF1 ZFP
construct targeting upstream 9-base pair target
site in VEGF promoter

<400> 15
Val Pro Ile Pro Gly Lys Lys Gln His Ile Cys His Ile Gln Gly
1 5 10 15

Cys Gly Lys Val Tyr Gly Thr Thr Ser Asn Leu Arg Arg His Leu Arg
20 25 30

Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly
35 40 45

Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His
50 55 60

Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met
65 70 75 80

Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys
85 90 95

Gly Gly Ser

<210> 16
<211> 298
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:VEGF3a ZFP
construct targeting downstream 9-base pair target
site in VEGF promoter

<220>
<221> CDS
<222> (2)..(298)
<223> VEGF3a

<400> 16

g gta ccc ata cct ggc aag aag aag cag cac atc tgc cac atc cag ggc 49
Val Pro Ile Pro Gly Lys Lys Gln His Ile Cys His Ile Gln Gly
1 5 10 15

tgt ggt aaa gtt tac ggc cag tcc tcc gac ctg cag cgt cac ctg cgc 97
Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg
20 25 30

tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt ggt 145
Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly
35 40 45

aaa cgc ttc acc cgt tcg tca aac cta cag agg cac aag cgt aca cac 193
Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His
50 55 60

acc ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc atg 241
Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met
65 70 75 80

cga agt gac gag ctg tca cga cat atc aag acc cac cag aac aag aag 289
Arg Ser Asp Glu Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys
85 90 95

ggg gga tcc
Gly Gly Ser 298

<210> 17
<211> 99
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:VEGF3a ZFP
construct targeting downstream 9-base pair target
site in VEGF promoter

<400> 17

Val Pro Ile Pro Gly Lys Lys Gln His Ile Cys His Ile Gln Gly
1 5 10 15

Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg
20 25 30

Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly
35 40 45

Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His
50 55 60

Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met
65 70 75 80

Arg Ser Asp Glu Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys
85 90 95

Gly Gly Ser

<210> 18
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:VEGF DNA target
site 1 recognition (top) strand

<220>
<221> protein_bind
<222> (11)..(19)
<223> VEGF DNA ZFP target site 1

<400> 18
catgcatagc ggggaggatc gccatcgat

29

<210> 19
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:VEGF DNA site 1
complementary (bottom) strand

<400> 19
atcgatggcg atcctcccg ctatgcatg

29

<210> 20
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:VEGF DNA
target site 3 recognition (top) strand

<220>
<221> protein_bind
<222> (11)..(19)
<223> VEGF DNA ZFP target site 3

<400> 20
catgcataatc gcggaggctt ggcatcgat

29

<210> 21
<211> 29
<212> DNA
<213> Artificial Sequence

<220>		
<223> Description of Artificial Sequence:VEGF DNA target		
site 3 complementary (bottom) strand		
<400> 21		
atcgatgcca agcctccgcg atatgcatg		29
<210> 22		
<211> 29		
<212> DNA		
<213> Artificial Sequence		
<220>		
<223> Description of Artificial Sequence:primer SPE7		
<400> 22		
gagcagaatt cggcaagaag aagcagcac		29
<210> 23		
<211> 26		
<212> DNA		
<213> Artificial Sequence		
<220>		
<223> Description of Artificial Sequence:primer SPEamp12		
<400> 23		
gtggtctaga cagctcgtca cttcgc		26
<210> 24		
<211> 28		
<212> DNA		
<213> Artificial Sequence		
<220>		
<223> Description of Artificial Sequence:primer SPE		
amp13		
<400> 24		
ggagccaagg ctgtggtaaa gtttacgg		28
<210> 25		
<211> 26		
<212> DNA		
<213> Artificial Sequence		
<220>		
<223> Description of Artificial Sequence:primer SPEamp11		
<400> 25		
ggagaagctt ggatcctcat tatccc		26
<210> 26		
<211> 83		
<212> DNA		
<213> Artificial Sequence		

<220>
 <223> Description of Artificial Sequence:sequence
 ligated between XbaI and StyI sites

<400> 26
 tcttagacaca tcaaaaaccca ccagaacaag aaagacggcg gtggcagcgg caaaaagaaaa 60
 cagcacatat gtcacatcca agg 83

<210> 27
 <211> 39
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:primer GB19

<400> 27
 gccatgccgg taccatacc tggcaagaag aagcagcac 39

<210> 28
 <211> 33
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:primer GB10

<400> 28
 cagatcgat ccacccttct tattctggtg ggt 33

<210> 29
 <211> 589
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:designed
 6-finger ZFP VEGF3a/1 from KpnI to BamHI

<220>
 <221> CDS
 <222> (2)..(589)
 <223> VEGF3a/1

<400> 29
 g gta ccc ata cct ggc aag aag aag cag cac atc tgc cac atc cag ggc 49
 Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly
 1 5 10 15

tgt ggt aaa gtt tac ggc cag tcc tcc gac ctg cag cgt cac ctg cgc 97
 Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg
 20 25 30

tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt ggt 145
 Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly

35	40	45	
aaa cgc ttc aca cgt tcg tca aac cta cag agg cac aag cgt aca cac			193
Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His			
50	55	60	
aca ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc atg			241
Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met			
65	70	75	80
cga agt gac gag ctg tct aga cac atc aaa acc cac cag aac aag aaa			289
Arg Ser Asp Glu Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys			
85	90	95	
gac ggc ggt ggc agc ggc aaa aag aaa cag cac ata tgt cac atc caa			337
Asp Gly Gly Ser Gly Lys Lys Gln His Ile Cys His Ile Gln			
100	105	110	
ggc tgt ggt aaa gtt tac ggc aca acc tca aat ctg cgt cgt cac ctg			385
Gly Cys Gly Lys Val Tyr Gly Thr Thr Ser Asn Leu Arg Arg His Leu			
115	120	125	
cgc tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt			433
Arg Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys			
130	135	140	
ggt aaa cgc ttc acc cgt tcg tca aac ctg cag cgt cac aag cgt acc			481
Gly Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr			
145	150	155	160
cac acc ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc			529
His Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe			
165	170	175	
atg cgt agt gac cac ctg tcc cgt cac atc aag acc cac cag aat aag			577
Met Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys			
180	185	190	
aag ggt gga tcc			589
Lys Gly Gly Ser			
195			
<210> 30			
<211> 196			
<212> PRT			
<213> Artificial Sequence			
<220>			
<223> Description of Artificial Sequence:designed			
6-finger ZFP VEGF3a/1 from KpnI to BamHI			
<400> 30			
Val Pro Ile Pro Gly Lys Lys Gln His Ile Cys His Ile Gln Gly			
1	5	10	15
Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg			
20	25	30	
Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly			

35	40	45
Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His		
50	55	60
Thr G1y Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met		
65	70	75
Arg Ser Asp G1u Leu Ser Arg His Ile Lys Thr His G1n Asn Lys Lys		
85	90	95
Asp Gly Gly G1y Ser G1y Lys Lys Gln His Ile Cys His Ile Gln		
100	105	110
Gly Cys Gly Lys Val Tyr G1y Thr Thr Ser Asn Leu Arg Arg His Leu		
115	120	125
Arg Trp His Thr G1y Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys		
130	135	140
Gly Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr		
145	150	155
His Thr G1y Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe		
165	170	175
Met Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His G1n Asn Lys		
180	185	190
Lys G1y G1y Ser		
195		

<210> 31
 <211> 42
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:JVF9 VEGF3a/1
 target oligonucleotide

 <400> 31
 agcgagcggg gaggatcgcg gaggcttggg gcagccgggt ag

42

<210> 32
 <211> 42
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:JVF10 VEGF3a/1
 target oligonucleotide complementary sequence

 <400> 32
 cgctctaccc ggctgccccca agcctccgcg atcctccccc ct

42

<210> 33
 <211> 25

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer JVF24

<400> 33
cgcggatccg cccccccgac cgatg 25
<210> 34
<211> 62
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:downstream
primer JVF25

<400> 34
ccgcaagctt acttgtcatc gtcgtccttg tagtcgctgc ccccaccgta ctcgtcaatt 60
cc 62

<210> 35
<211> 7
<212> PRT
<213> Simian virus 40

<220>
<221> PEPTIDE
<222> (1)..(7)
<223> SV40 large T antigen nuclear localization sequence
(NLS)

<400> 35
Pro Lys Lys Lys Arg Lys Val
1 5

<210> 36
<211> 61
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:segment from
EcoRI to KpnI containing Kozak sequence including
initiation codon and SV40 NLS

<400> 36
gaattcgctt ggcgcaccat ggcccccggaa aagaagagggat ccatggggta 60
c 61

<210> 37
<211> 187
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:segment from
KpnI to XhoI containing BamHI site, KRAB-A box
from KOX1, FLAG epitope and HindIII site

<400> 37
ggtacccggg gatcccgac actggtgacc ttcaaggatg tatttggta cttcaccagg 60
gaggagtgga agctgctgga cactgctcag cagatcgatg acagaaatgt gatgctggag 120
aactataaga acctggtttc cttgggcagc gactacaagg acgacgatga caagtaagct 180
tctcgag 187

<210> 38
<211> 277
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:inserted
fragment from BamHI to HindIII sites

<400> 38
ggatccggcc ccccgaccga tgcgcgcctg ggggacgagc tccacttaga cggcgaggac 60
gtggcgatgg cgcatgccga cgcgcgtac gatttcgatc tggacatgtt gggggacggg 120
gattccccgg ggccgggatt taccccccac gactccggcc cctacggcgc tctggatatg 180
gccgacttcg agttttagca gatgtttacc gatgcccttgc gaatttagca gtacgggtggg 240
ggcagcgact acaaggacga cgatgacaag taagctt 277

<210> 39
<211> 118
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:sequence
replacing NLS-KRAB-FLAG with NLS-FLAG only

<400> 39
gaattcgcta gcgccaccat ggcccccgg aagaagagga aggtggaaat ccatgggta 60
ccggggatg gatccggcag cgactacaag gacgacgatg acaagtaagc ttctcgag 118

<210> 40
<211> 204
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:insert into
MluI/BglII sites of pGL3-Control to create
pVFR1-4x

<400> 40
acgcgttaagc ttgctagcga gcggggagga tcgcggaggc ttggggcagc cgggttagac 60
gagcggggag gatcgccggag gcttggggca gccgggtaga gcgagcgggg aggatcgccg 120
aggcttgggg cagccggta gagcgagcgg ggaggatcgc ggaggcttgg ggcagccggg 180
tagagcgctc agaagcttag atct 204

<210> 41
<211> 4
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: "D-able" site motif

<400> 41
nngk 4

<210> 42
<211> 4
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: D-able site subtype

<400> 42
nngg 4

<210> 43
<211> 4
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: D-able site subtype

<400> 43
nngt 4